

GE Healthcare

Ettan DALT*twelve* System

Second-dimension gel electrophoresis

User Manual



Important user information

All users must read this entire manual to fully understand the safe use of Ettan DALTtwelve system

Important!

Ettan DALTtwelve system is intended for research use only, and should not be used in any clinical or in vitro procedures for diagnostic purposes.

Safety notices

This manual contains warnings and cautions concerning the safe use of the product. See definitions below.

WARNING!



The WARNING! sign highlights instructions that must be followed to avoid personal injury. Do not proceed until all stated conditions are clearly understood and met.

CAUTION!

The CAUTION! sign highlights instructions that must be followed to avoid damage to the product or other equipment. Do not proceed until all stated conditions are met and clearly understood.

Note

The Note sign is used to indicate information important for trouble-free and optimal use of the product.

CE Certification

This product meets all requirements of applicable CE-directives. A copy of the corresponding Declaration of Conformity is available on request.

The **CE** symbol and corresponding declaration of conformity, is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other CE-marked GE Healthcare instruments, or
- connected to other products recommended or described in this manual, and
- used in the same state as it was delivered from GE Healthcare except for alterations described in this manual.

Recycling



This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.

This is a Class A product. In a domestic environment, it might cause radio interference, in which case the user might be required to take appropriate measures.



WARNING! All repairs should be done by personnel authorized by GE Healthcare. Do not open any covers or replace any parts unless specifically stated in the instructions.



WARNING! The system must be connected to a grounded power supply socket.



WARNING! If liquid is spilled on the equipment, the power supply shall be disconnected immediately.



WARNING! The system must not be opened by the user. It contains high voltage circuits that can give a lethal electrical shock.



WARNING! Only use mains cables delivered or approved by GE Healthcare.



WARNING! Do not block the rear panel of the system. The mains power switch must always be easy to access.



WARNING! When using hazardous chemicals, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the chemicals used. Follow local regulations and instructions for safe operation and maintenance of the system.

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1 Ettan DALT*twelve* System

In 2-D electrophoresis, proteins are separated according to isoelectric point by isoelectric focusing, most reliably on immobilized pH gradient (IPG) strips using the IPGphor™ IEF System. The second-dimension electrophoresis separates the proteins on the basis of their molecular mass using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The Ettan™ DALT*twelve* System is designed to handle multiple large second dimension gels in a simple, efficient, and reproducible manner.

1.1 Ettan DALT*twelve* System components

- 12-slot vertical slab separation unit
- 200 W, Power Supply/Control Unit
- gel caster
- gel casting cassettes
- gradient maker with peristaltic pump.

1.1.1 Separation Unit



Fig 1-1 Ettan DALT*twelve* Separation Unit.

The Ettan DALT*twelve* Separation Unit accommodates up to twelve 25.5 × 20.5 cm slab gels for separation under identical conditions. A sample, focused in an IPG strip, is placed on the cathodic surface of a slab gel and sealed with agarose. Up to 12 gels are inserted into the separation unit through the buffer seal slots flanked by rubber gaskets. Any unused slots are filled with the blank cassette inserts. The buffer seal is an effective current and liquid barrier.

The platinum wire cathode is attached to the underside of the lid, and the platinum wire anode is in the lower tank. Safety interlocks prevent the application of power to the separation unit unless the lid is closed properly and the pump valve is in the circulate position. The lid is easily removed for cleaning by sliding it off its hinges.

A pump located in the lower chassis of the unit circulates the buffer, pumping it up into the main chamber on the left side, between the cassettes, down the right side and over the internal heat exchanger before returning to the pump. The default setting for the pump is auto; the pump will come on only when a run is started. Turning the lever at the back of the unit from circulate to drain drains the tank. The temperature is controlled by Peltier modules attached to the heat exchanger beneath the tank.

1.1.2 Power Supply/Control Unit

The Ettan DALTtwelve System is controlled from the Power Supply/Control Unit (see Fig 1-2). The unit supplies a maximum power output of 200 W with a maximum of 600 V or 1 A. The temperature control range is 10°C to 50°C.



WARNING! The equipment may not be plugged into the mains power outlet unless all the cables between the control unit and separation unit are properly connected. The high voltage connector on the control unit has a voltage of 600 volts DC.

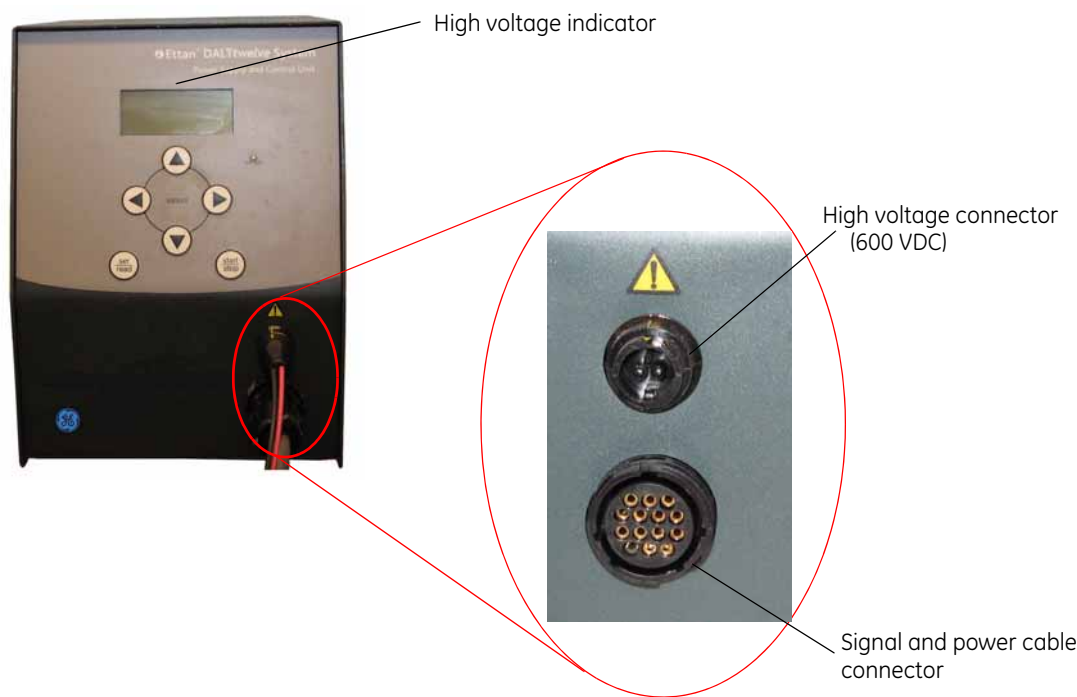


Fig 1-2 Front of Power Supply/Control Unit.

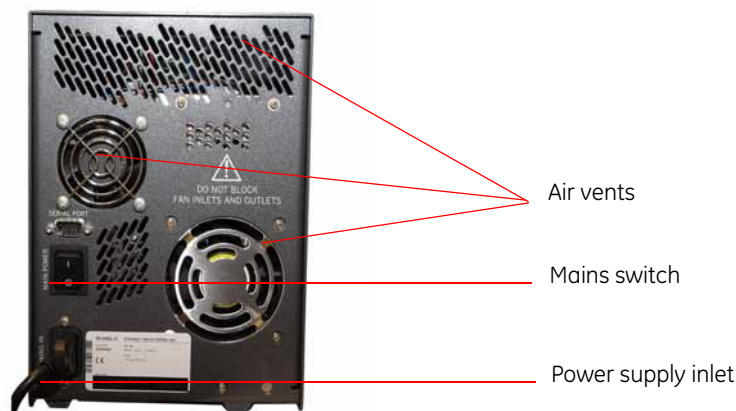


Fig 1-3 Rear of Power Supply/Control Unit

1.1.3 Electrical connections

The power supply/control unit provides the separator with 600 VDC from the upper connector. The lower connector provides power and control to the separator. See Fig 1-2.

On the rear side of the power supply/control unit there is a power inlet to which the power supply cord is connected and an RS232 port. See Fig 1-3.

1.1.4 Gel Caster

The Ettan DALTwelve Gel Caster holds up to fourteen 1.0 mm or thirteen 1.5 mm gel cassettes, with separator sheets, for casting homogenous or gradient gels (see Fig 1-4). Fewer gels can be cast at one time by using blank cassette inserts to occupy unneeded volume. The removable faceplate and separator sheets simplify loading and unloading of cassettes from the unit. The unit also has removable tilt support legs that allow the caster to be tipped back for more convenient loading of gel casting cassettes.



Fig 1-4 Gel Caster

1.1.5 Gel Casting Cassettes

The gel casting cassettes are pre-assembled. Two glass plates are joined along one edge by a hinge strip of silicone rubber, and the vinyl side spacers (1.0 mm or 1.5 mm thick) are glued in place. To complete assembly, close the two plates like a book and press the plates together over the length of the spacer. Gels are removed by opening the book after the run and carefully lifting out the gel slab. Care must be taken to ensure that the gel does not adhere to the spacers and tear during removal. The cassette is cleaned as a unit and can be stood upright to dry. The cassettes can be cleaned in an automatic dishwasher. Cassettes are 27 × 22 cm and produce a gel about 25.5 × 20.5 cm. A 1.0 mm thick gel has a volume of approximately 52 ml and a 1.5 mm thick gel has a volume of approximately 78 ml.



Fig 1-5 Gel Casting Cassette

1.2 Important information

IMPORTANT! Ettan DALTtwelve System is intended for laboratory use only, not for clinical or *in vitro* use, or for diagnostic purposes.



WARNING! When using hazardous chemicals, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the chemicals used. Follow local regulations and instructions for safe operation and maintenance of the system.



WARNING! In case of an emergency situation, the mains plug must always be accessible and easy to disconnect.



WARNING! Always disconnect the power supply cord from the control unit before disconnecting the cables and cords between the separation unit and the control unit.



WARNING! Take care when handling glass sheets to avoid injury caused by sharp edges.

- Connect the instrument to a properly grounded electrical outlet.
- The safety lid must be firmly in place and the pump valve set to circulate before power can be applied.
- Stop the run before opening the safety lid.
- Rinse and flush the tank and pumping system with distilled or deionized water before and after use.
- Always disconnect the power cord before maintenance or repair.
- Do not run the circulation pump if the separation unit is empty.
- Do not operate with buffer temperature above 50°C. All plastic parts are rated for 50°C continuous duty.
- Turn the pump on during electrophoresis to minimize heating. Overheating will cause irreparable damage to the unit.
- For runs near the lower temperature limits (~10 to 15°C), it may be necessary to operate the unit in a cold room, especially in laboratories where the ambient temperature is above 25°C.
- Do not autoclave or boil this unit or any of its parts.
- Make sure there are no loose parts or debris inside the equipment.
- Use care when lifting and moving the separation unit. It is best to move the unit when empty.
- When filled with glass plates and gel solutions, the casting unit is very heavy. Use caution when moving or lifting the caster.
- If this equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

- Only accessories and parts approved or supplied by GE Healthcare may be used for operating, maintaining and servicing this product.



WARNING! To avoid risk of electrical shock, ensure that all electrode contacts are clean and dry and there is no excess liquid on the upper rim of the separation unit. Inspect the cables for damage before use of the instrument and contact Technical Support if any damage is apparent.

1.3 Specifications

Ettan DALT*twelve* Electrophoresis Unit

Gel capacity	12 gels
Electrophoresis buffer volume	10 l
Dimensions (h × w × d)	54 × 46 × 51 cm, lid closed 74 × 46 × 51 cm, lid open
Weight	24.5 kg
Maximum temperature	50°C
Environmental operating conditions	Indoor use, 4°C to 40°C Humidity up to 90% Altitude to 2000 m
Buffer circulation pump rate	~10 l/min
Ettan DALT<i>twelve</i> Power Supply/Control Unit	
Maximum output power	200 W
Maximum output voltage	600 V DC
Maximum output current	1.00 A
Temperature control range	10°C to 50°C
Weight	6.35 kg
Dimensions (h × w × d)	33 × 26 × 19 cm
Environmental operating conditions	Indoor use, 4°C to 40°C Humidity up to 90% Altitude to 2000 m
Installation category	II
Pollution degree	2
Supply voltage	110 to 120 VAC, 50 to 60 Hz 220 to 240 VAC, 50 to 60 Hz
Max. Power Consumption	650 W
Product and safety certifications*	EN61010-1, IEC61010-1, UL61010-1, CSA C22.2 1010.1, CE Certified
Complies with EMC standard	EN61326

Ettan DALT*twelve* Gel Caster

Gel capacity	14 for 1.0 mm thick gel 13 for 1.5 mm thick gel
Acrylamide solution volume (total)	950 ml for 1.0 mm thick gel 1200 ml for 1.5 mm thick gel
Dimensions (h × w × d)	26 × 21 × 36 cm
Weight	(empty) 5 kg (loaded) 19 kg

1.0 mm Gel Casting Cassette

Cassette dimensions (h × w × d)	21.7 × 27.6 × 0.70 cm
Slab gel dimensions (h × w × d)	20.5 × 25.5 × 0.10 cm

1.5 mm Gel Casting Cassette

Cassette dimensions (h × w × d)	21.7 × 27.6 × 0.75 cm
Slab gel dimensions (h × w × d)	20.5 × 25.5 × 0.15 cm

Gradient Maker

Dimensions (h × w × d)	54 × 19 × 18 cm
Maximum gradient volume	2 l
Weight	5.4 kg

Peristaltic Pump for Gradient Maker

Supply voltage	100 to 118 VAC, 50 to 60 Hz 220 to 237 VAC, 50 to 60 Hz
Max. Power Consumption	37 W
Max input current 115 V	1.5 A (SC output)
Max input current 230 V	0.9 A (SC output)
Weight	4.1 kg
Dimensions (h × d × w)	13.5 × 18 × 22 cm
Environmental operating conditions	Indoor use: 0°C to 40°C Humidity: 10% to 90%
Installation category	II
Pollution degree	2
Product certifications*	EN61010-1, UL508, CSA C22.2, No.14, IEC 61010
Complies with EMC standard	EN61326
IP class	IP22

2 Preparing the gel caster

Unpack the equipment and take care when moving the equipment into position. Use the handles to lift and position the units on a level and stable surface.

IMPORTANT! Make sure that the supply cord and the power outlet are easily accessible and that no ventilation openings in the enclosure are blocked in any way.



WARNING! Make sure that there are no loose parts or debris inside the equipment.

Set up the gel caster near a sink, in a tray, or on a drainboard so that any liquid that may overflow from the unit or drain out of it during pouring or disassembly can be easily contained.

The Ettan DALT*twelve* Gel Caster (see Fig 2-1) can accommodate up to fourteen 1.0 mm or thirteen 1.5 mm gel cassettes with separator sheets between them. If you are not planning to cast a full set of gels, use the blank cassette inserts, with separator sheets in between (provided with the separation unit) to occupy the extra space.

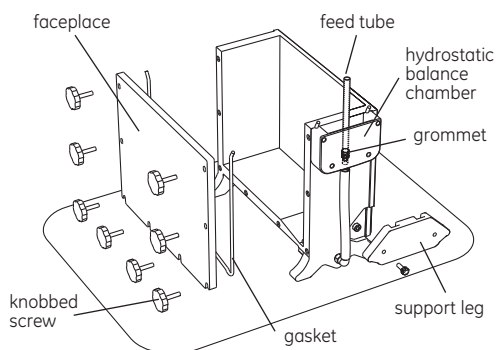


Fig 2-1 Exploded view of caster.

Gel labels, for easy indexing of gels and samples, can be placed in the cassettes at any time during the assembly of the caster.

- 1 Check that the caster is level. Remove the faceplate and tip the caster back so that it rests on the removable tilt support legs. If casting gradient gels, place the triangular sponge in the base of the V-shaped feed channel; otherwise, it may be omitted (Fig 2-2).



Fig 2-2 Gel caster with gel cassette, tipped back.

2 Preparing the gel caster

- 2 Start filling the gel caster by placing a separator sheet against the back wall to make it easier to remove the last cassette from the unit after polymerization. Fill the caster by alternating cassettes with separator sheets. The rubber hinge should be on the left side of the caster, with the offset end of the cassette up. End with a separator sheet then use the thicker spacer sheets to bring the level of the stack even with the edge of the caster.
- 3 Lubricate the foam gasket with a light coating of GelSeal compound to help ensure a liquid-tight seal. Place the gasket in the groove on the faceplate. Avoid stretching the gasket by seating it from the ends toward the middle.
- 4 Turn four black-knobbed screws into the four threaded holes across the bottom until they are well engaged (two to three full turns). Carefully place the faceplate onto the caster with the bottom slots resting on their respective screws. Screw the four remaining black-knobbed screws into the holes at the sides of the faceplate and tighten all eight evenly. Be sure the sealing gasket is compressed evenly by the faceplate and forms a tight seal with the caster. Do not overtighten the screws (see Fig 2-3).



Fig 2-3 Gel caster filled and level.

- 5 Insert the end of the rigid plastic feed tube, supplied with the gel caster, into the rubber grommet in the floor of the hydrostatic balance chamber on the side of the caster. The feed tube must be snug so that there is no leakage from the balance chamber into the caster. The feed tube should be connected with flexible tubing directly to either a peristaltic pump or a funnel (Fig 3-1).

3 Casting homogeneous gels



WARNING! Acrylamide is a neurotoxin. Never pipette by mouth and always wear protective gloves when working with acrylamide solutions, IPG strips, or surfaces that come into contact with acrylamide solutions.

- 1 Be sure the entire gel casting system is clean, dry, and free of any polymerized acrylamide.
- 2 Prepare a sufficient volume of gel overlay solution (water-saturated n-butanol). You need 1 ml of overlay for each 1.0 mm cassette and 1.5 ml for each 1.5 mm cassette.
- 3 Make up 100 ml of displacing solution (0.375 M TrisCl pH 8.8, 50% (v/v) glycerol, bromophenol blue).
- 4 For a full 14-gel 1.0 mm cassette set, make up 0.9 l of acrylamide gel stock solution without adding the 10% (w/v) ammonium persulphate (APS) or 10% (v/v) N,N,N',N'- tetramethylethylenediamine (TEMED). For a full 13-gel 1.5 mm cassette set, make up 1.2 l of acrylamide gel stock solution as above. This amount of gel solution will provide you with sufficient volume to cast gels using either a funnel or a peristaltic pump.
- 5 Assemble the gel caster as described in the preceding section; the caster should be placed on a level bench or on a leveling table so that gel tops are level. The white triangular sponge may be omitted.
- 6 Connect the feed tube to either a funnel held in a ring-stand above the top of the gel caster (about 30 cm) or a peristaltic pump. Insert the other end of the feed tube into the grommet in the bottom of the balance chamber (see Fig 3-1).

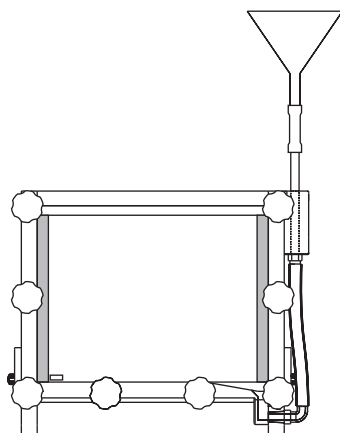


Fig 3-1 Gel caster, in use.

- 7 Fill the balance chamber with 100 ml of the displacing solution.
- 8 Add the appropriate volumes of APS and TEMED only when ready to pour the gels, not before. Once these two components are added, polymerization begins and the gel solution should be completely poured within 10 min.
- 9 Pour the gel solution into the funnel, taking care to avoid introducing any air bubbles into the feed tube. If a peristaltic pump is being used, the flow rate should be increased slowly to the desired speed to avoid introducing any air bubbles into the feed tube.

3 Casting homogeneous gels

- 10 Use the peristaltic pump to pump the gel solution into the caster until it is about 1 to 2 cm below the final desired gel height. Stop the flow of acrylamide and remove the feed tube from the balance chamber grommet. Once the feed tube is removed, the dense displacing solution flows down the connecting tube, filling the V-well and sloped trough at the bottom of the caster. The remaining acrylamide solution is forced into the cassettes to the final gel height. The amount of gel solution required will be 800 to 850 ml for gels 1.0 mm thick and ~1150 ml for 1.5 mm thick gels.
- 11 Immediately pipette 1 ml of water-saturated butanol onto each gel. If you are using a peristaltic pump to pour the gels, rinse the gel solution from the pump before it begins to polymerize.
- 12 Allow the homogeneous gels to polymerize for at least 1 h before disassembling the caster.

4 Casting gradient gels

4.1 Preparing for gradient gel casting

Successful gradient gel casting requires planning, timing, and practice. A full cast with the Ettan DALT^{twelve} Gel Caster requires approximately 0.9 to 1.2 l of acrylamide stock. Polymerization begins as soon as TEMED and APS are added to the acrylamide stock. At this point there is no time to adjust the gradient maker divider, or the cassettes and separators in the gel caster. To familiarize yourself with the gel caster and gradient maker before casting gels, we recommend setting up the unit and configuring the gradient divider as described below. Follow the gradient pouring procedure, substituting water for the appropriate volume of light solution, and a mixture of glycerol and water for the appropriate volume of heavy solution. When the angle of the gradient divider is adjusted correctly and you are comfortable with the gel casting procedure, clean all parts of the caster and gradient maker, including sponges, separator sheets and filler blocks, with a solution of mild detergent, followed by a deionized water rinse.

4.2 Configuring the gradient divider

You can adjust the shape of the gradient divider to suit the number of gels to be cast and the shape of the gradient you want. When casting a full tank of gels, the angle of the adjustable divider to the floor of the gradient maker should be about 40° (see Fig 4-1). For fewer gels, decrease the divider angle. Whatever angle you use, a straight divider gives a linear gradient.

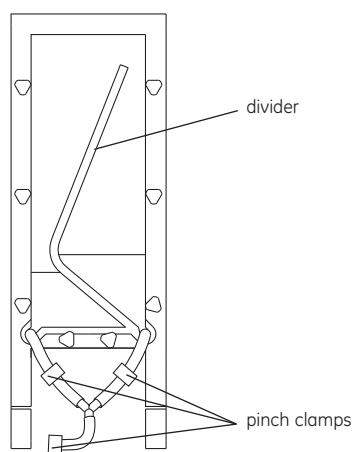


Fig 4-1 Configuration of the gradient maker.

- 1 Determine the amount of gel solution needed to cast the desired number of gels.
- 2 Loosen the faceplate screws on the empty gradient maker to adjust the gasket angle. Refer to the divider configuration in Fig 4-1 as a guideline for the angle you should use. Pull the divider slightly to help move it into position. Use the red adjuster rod provided with the gradient maker to push the gradient divider down.
- 3 For a linear gradient, straighten the divider, with its upper end almost touching the left wall at the height determined by the volume of water.
- 4 To make a funnel for introducing heavy solution into the left side, bend the remaining top of the divider to the right.
- 5 Tighten the faceplate screws and close the pinch clamps on the outflow tubing at the bottom of the gradient maker before adding water or gel solutions.

- 6 Using water in place of gel solution, put half of the required volume in the right chamber and the other half in the left chamber.
- 7 Adjust the angle of the gradient divider so that the level of liquid in the “heavy”, or left, chamber is about 2 cm below the level of liquid in the “light” chamber. You can use tape or wax pencil on the outside of the gradient maker to mark the angle of the gradient divider.
- 8 Open the pinch clamps to remove the water, or pour the water out of the top of the gradient maker. Repeat this procedure whenever the required volume of acrylamide solution changes as a result of changing the number of gels you are casting.

4.3 Calibrating the peristaltic pump

Install the pump head and tubing on the pump controller as directed in the User Manual supplied with the pump. Calibrate the pump flow rate before the first use and after every 10 to 20 uses, to ensure proper flow rates. This calibration procedure assumes a desired flow rate of 335 ml/min for 14 gels.

Example. You want to cast a full tank of gels and determine that the flow rate is 500 ml/min when you set the flow rate at 3. For a flow rate of 335 ml/min:

$(335 \div 500) 3 = 2$ = the appropriate flow rate setting to deliver 335 ml/min.

- 1 Place the inlet side of the tubing in a beaker that contains 1 l of water.
- 2 Place the outlet side of the tubing in a 1 l graduated cylinder.
- 3 Set the flow speed at 3 on the dial and start the pump.
- 4 Stop the flow of liquid after exactly 2 min.
- 5 Measure the water in the outlet cylinder and divide by 2 to determine the flow in ml/min.
- 6 To determine the appropriate flow setting, divide the desired flow rate by the flow rate in step 5; multiply this result by the flow speed used in step 3.

4.4 Casting gradient gels

The gradient maker is a simple unit with two chambers that are defined by an adjustable silicone rubber gasket clamped between two acrylic plates. You can modify the shape of the gel gradient by adjusting the movable divider. Solutions flow out of the two chambers in proportion to the relative widths at the surface of the liquid, join at the Y-connector, and then are thoroughly mixed in an in-line “bow-tie” mixer that has no moving parts. Three pinch clamps control the flow at the exits from the light (a) and heavy (b) chambers, and after the mixer (c) to control flow into the peristaltic pump (Fig 4-2).

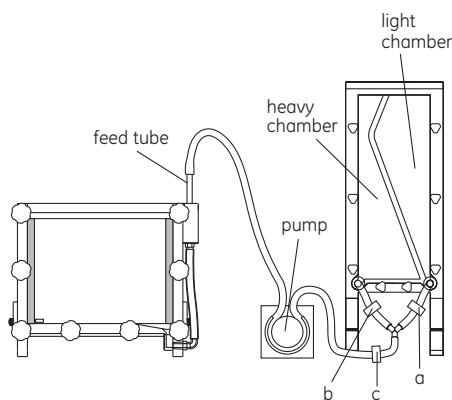


Fig 4-2 Pump connected to gradient maker.

A gradient gel results from using two gel solutions of different acrylamide concentrations and densities—a light solution and a heavy solution. The heavy gel solution contains glycerol. During the gradient pouring procedure, the mixing ratio of heavy solution to light solution gradually increases, with the heavier solution underlaying the light solution. This generates a downward gradient of increasing gel percentage. To ensure balanced flow, when the gradient maker is filled with equal volumes on each side of the divider, the height of the heavy gel solution in the gradient maker should be 1 to 2 cm less than the height of the light solution. Under these conditions, the two solutions are in hydrostatic equilibrium, see Section 4.2. Hydrostatic equilibrium can also be achieved by using equal masses, instead of volumes, of the heavy and light solutions.

4.5 Gradient casting setup



WARNING! Acrylamide is a neurotoxin. Never pipette by mouth and always wear protective gloves when working with acrylamide solutions, IPG strips, or surfaces that come into contact with acrylamide solutions.

- 1 Be sure the entire gel casting system is clean, dry, and free of any polymerized acrylamide. Place the white sponge in the base of the V-shaped feed channel of the caster. The caster should be placed on a level bench to ensure that the gels and gradients are even and level.
- 2 Configure the gradient divider for the number of gels you are casting. If necessary, calibrate the gradient pump flow rate. See "Calibrating the peristaltic pump" on page 9.
- 3 Be sure that the faceplate screws on the gradient maker are fingertight and all the gradient maker lines are clamped off. There are three clamps: one coming from each chamber and one after the bow-tie mixer. Close all three.
- 4 Prepare a sufficient volume of gel overlay solution (water-saturated n-butanol). You need 1 ml of overlay for each 1.0 mm cassette, and 1.5 ml for each 1.5 mm cassette.
- 5 Make up 100 ml of displacing solution. See Section 10.2.
- 6 Make up the gel acrylamide solutions from the stock mixes, but do not add the 10% ammonium persulphate (APS) and 10% N,N,N',N'-tetramethylethylenediamine (TEMED). See Section 10.2.

4.6 Pouring gel solutions for gradient gels

- 1 Prepare the gel caster, as described on page 6, placing gel labels in each cassette.
- 2 When you are ready to cast the gels, add the APS and TEMED and mix each gel solution thoroughly. Vary the amount of TEMED added to control the rate of polymerization. Once these reagents are added, polymerization begins. You have about 10 min to cast the gradient before the gels begin to solidify at the top. Work rapidly and carefully.
- 3 Pour the light solution into the right side of the gradient maker (the chamber that is narrower at the bottom —“Light in right”).
- 4 Fill the tubing between the light and heavy chambers with light solution. Carefully open the clamp on the light chamber exit tube (a) and then very slowly open the heavy chamber exit tube clamp (b). Allow light solution to fill the tube coming from the light chamber all the way to the Y-connector and back up to the point at which the heavy tube enters the heavy chamber. Fill the entire tube with light solution (no bubbles), but do not allow light solution into the heavy chamber itself (4-3 *Priming the gradient marker with light solution.*)

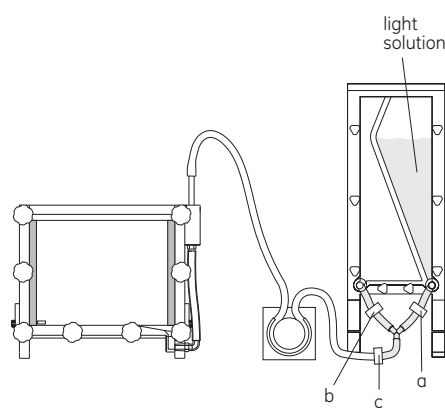


Fig 4-3 Priming the gradient marker with light solution.

- 5 Close both clamps again. All three clamps should now be closed.
- 6 Add the heavy solution to the heavy (left) chamber (the chamber that is wider at the bottom) until the liquid level reaches a point about 2 cm below the level of light solution in the adjacent chamber.
- 7 Load the balance chamber on the side of the caster with 75 ml of displacing solution (see Fig 4-4). The feed tube should be seated securely in the grommet seal to prevent leakage of displacing solution into the caster.

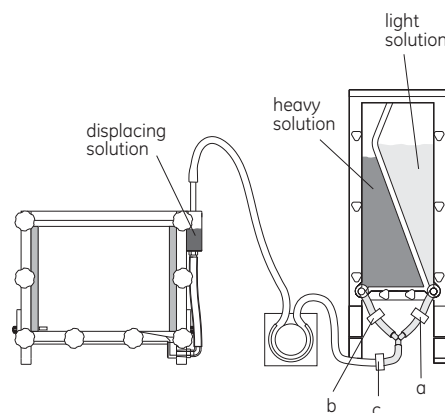


Fig 4-4 Properly filled caster and gradient marker.

- 8 Open the clamp below the mixer (c) to start flow to the gel caster via the peristaltic pump.
- 9 Carefully open the clamp on the light chamber exit tube (a) and turn on the pump to bring a small amount of solution into the caster. Light solution should begin to flow through the feed tube and mixer toward the caster. At this point a small amount of light solution can enter the caster.
- 10 When the light solution level in the gradient maker falls to about 1 cm above the level of the heavy solution, open the heavy chamber exit tube clamp (b).
- 11 Watch the gradient enter the caster.
- 12 When the caster is filled to about 1 to 2 cm below the final desired gel height or the gradient maker is empty, turn off the pump and close the feed tube clamp (c). Stop the pump before air enters the feed tube.
- 13 Pull the feed tube out of the balance chamber grommet. Place the end in a waste container to collect the excess polymerizing acrylamide. As soon as the feed tube is removed, the dense blue displacing solution flows down the connecting tube to the unit. It should completely fill the V-well and the sloped trough at the bottom of the caster. If the V-well is not completely filled and the level of gel in the cassettes is more than 1 cm below the top of the cassettes, you can add up to 25 ml more displacing solution to the balance chamber. The gradient is now in hydrostatic equilibrium in the unit, ready to polymerize (see Fig 4-5).

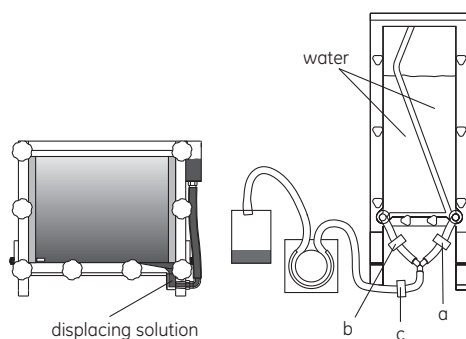


Fig 4-5 Finished cast.

- 14 Immediately pipette gel overlay solution (water-saturated n-butanol) onto each gel. Apply 1 ml of overlay for each 1.0 mm cassette and 1.5 ml for each 1.5 mm cassette. Allow gels to polymerize at least 2 h.
- 15 Quickly reopen clamp (c) and restart the pump to empty the gradient maker of any excess polymerizing acrylamide. Collect the excess in a waste container. Dispose of unpolymerized acrylamide according to applicable safety guidelines.
- 16 Rinse the gradient maker well to prevent polymerization within the tubing lines. Place the feed tube in a larger waste vessel or a sink drain. Pour a liter of water into each chamber of the gradient maker and open all clamps.
- 17 Start the pump to flush the system. Flush another 2 l of water through the gradient maker and tubing.

5 Polymerization

Allow nongradient gels to polymerize for at least 1 h; allow gradient gels 2 h to polymerize. Gradient gel polymerization should proceed from the top down. You can observe this through the front and sides of the caster. The level of the dense displacing solution falls farther as the gels contract upon polymerization.

6 Unloading the gel caster

- 1 Make sure the caster is either near a sink or on a tray so that any liquid leaking out can be contained.



WARNING! When handling the equipment manually the power shall be switched off. If there is a risk that liquid may be splashed or spilled on the equipment, then the power supply cord must be disconnected while handling the equipment.

- 2 Remove the front of the gel caster by loosening and removing the black-knobbed screws.
- 3 Carefully unload the cassettes from the unit by pulling forward on the separator sheets.
- 4 Rinse the top surface of each gel with distilled water to remove the butanol and any unpolymerized acrylamide. Remove the separator sheet if still attached and rinse the glass cassettes with water to remove any acrylamide adhering to the glass plates.
- 5 Examine the gels for polymerization defects and discard any unsatisfactory gels.
- 6 Store the good gels in an airtight container at 4°C with a small amount of gel storage solution to keep the gels from drying out.
- 7 Rinse the gel caster and all tubing with mild detergent, then rinse thoroughly with deionized water. Clean the separator and spacer sheets with a mild detergent and rinse with deionized water.

7 Electrophoresis

7.1 Installing the power supply/control unit



WARNING! Never connect the power supply cable before all other cables are connected and have been checked for damage.

The unit should be placed close to a sink for easy rinsing and draining. It must be placed on a stable support such as a bench or sturdy table.

The ventilation openings must not be blocked and the power switch and outlet must be easily accessible.

A length of rubber or vinyl tubing sufficiently long to reach a sink should be attached to the drain port on the back of the unit before use. The unit should not be placed on bench paper or any other material that might be pulled in by the air intake fans, as any hindrance to air circulation will reduce the cooling capacity.

7.2 Programming the power supply/control unit

The control unit has four programmable parameters: Run Type, Timer, Pump, and Temperature. When the unit is turned on, the default settings of constant power, continuous run, auto pump, and 25°C are shown on the display. The *set/read* button toggles the controller between the set and read modes. The *start/stop* button starts and stops the execution of the programmed electrophoresis run. The ◀ and ▶ buttons move the cursor between run parameters, and the ▲ and ▼ buttons change the settings of the parameters (7-1 *Controller interface in programming mode*).



Fig 7-1 Controller interface in programming mode.

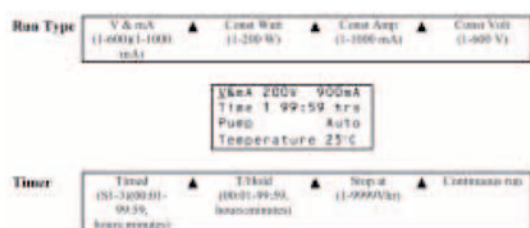
Sample program Twelve gels electrophoresed at 180 W, constant power, and 25°C with a 5 W/gel entry phase. The second phase is extended longer than required to ensure that the dye front runs off the gel.

Step #1

Const Watt	60 W
Time 1	00:45 hrs
Pump	Auto
Temperature	25°C

Step #2

Const Watt	180 W
Time 2	04:00 hrs
Pump	Auto
Temperature	25°C



Run Type determines the method of power regulation. The options for run type are constant power, constant current, constant voltage, and crossover mode. In crossover mode the voltage and current limits for the run are set manually, instead of using the instrument defaults of 600 V and 1 000 mA. As the run progresses, the power supply operates in the mode that is limiting.

Timer controls the duration of the electrophoresis run. The options are continuous run, timed step, timed and hold, and volt-hours. Up to three timed steps of up to 100 h each can be programmed using the timed step mode. With timed step mode, all the steps must have the same run type.

Pump controls buffer circulation through the separation unit. The options for the pump are on, off, and auto. Auto mode activates the pump only when power is applied.

Temperature controls the cooling or heating of the buffer in the separation unit. The temperature range is from 10°C to 50°C. On the display, cooling is indicated by and heating is indicated by. The pump must be on to properly cool or heat buffer in the tank. The lower temperature limit is a function of the ambient temperature and the power reading. To reach the lower temperature limit (10°C) or in laboratory environments where the ambient temperature is elevated, it may be necessary to place the unit in a cold box or cold room.

7.3 Preparing the electrophoresis unit for lab cast gels

The Ettan DALTtwelve Electrophoresis Unit requires a total volume of about 10 l of electrophoresis buffers to fill both the upper and lower chambers. For lab cast Laemmli gels, the lower buffer chamber is filled with 7.5 l of 1 × SDS electrophoresis buffer while the upper buffer chamber is filled with 2.5 l of 2 × SDS electrophoresis buffer. With the full volume of the lower buffer in the unit, the liquid buffer serves as a lubricant for inserting the glass cassettes through the buffer seal. Lubrication of the cassettes and the rubber surfaces of the buffer seal is vital when loading the unit. Forcing dry cassettes through the slots can severely damage the seal. The electrophoresis buffer can be made within the tank using the internal circulating pump to mix the solution.



WARNING! Do not overfill the tank. Spillage can cause shortcircuiting of internal circuits and the breakdown of electrical isolation which increases the risk of electrical shock.

- 1 Before filling the tank, turn the pump valve to circulate (see Fig 7-2).



Fig 7-2 Circulation valve.

- 2 Fill the electrophoresis tank to the 7.5 l fill line with 1× SDS electrophoresis buffer. Turn on the pump/control unit, and turn the pump on. If the pump fails to begin circulating buffer immediately, the pump must be primed; turn the pump valve to drain then back to circulate while the pump is on.
- 3 On the control unit, adjust the temperature to the desired setting.

7.4 Preparing second-dimension gels: equilibration and loading

For a detailed description of the components of the SDS equilibration solution and the equilibration process, please consult *2-D Electrophoresis: Using Immobilized pH Gradients* (80-6429-60).



WARNING! Take care when handling glass sheets. You risk cutting yourself on sharp edges.

- 1 Prepare SDS equilibration buffer. Just prior to use, add DTT to the buffer to a concentration of 1% (w/v).
- 2 Place the IPG strips in individual tubes with the support film toward the wall.
- 3 Add 5 to 10 ml of the DTT-containing solution to each tube. Typically, two 18-cm strips can be equilibrated with 10 ml of buffer.
- 4 Incubate the strips for 10 to 15 min with gentle agitation. Do not overequilibrate, as proteins can diffuse out of the strip during this step.
- 5 Second equilibration (optional): Prepare SDS equilibration buffer with iodoacetamide added to 2.5% (w/v) and equilibrate the strips with this solution, as in steps 3 to 4.
- 6 Before equilibration is completed, prepare the gel cassettes for loading by rinsing the top of the gel with deionized water and draining. Before loading the IPG strips, make sure that the gel surface and plates are dry.



Fig 7-3 IPG strip being positioned on cassette.



Fig 7-4 IPG strip begin seated against slab gel.

- 7 Lay the prepared gel flat on a clean surface.

- 8 Using forceps, remove the equilibrated IPG strip from the equilibration solution and rinse with fresh SDS electrophoresis buffer.
- 9 Holding one end of the IPG strip with forceps, carefully draw it across the long gel plate until the strip is completely on the glass plate and centered (see Fig 7-3). Using a thin plastic spatula, ruler, or spacer, push against the plastic backing of the IPG strip—not the gel itself—and slide the strip between the two glass plates and down into contact with the surface of the slab gel (*7-4 IPG strip begin seated against slab gel.*). The strip should just rest on the surface of the gel. Avoid trapping air bubbles between strip and the slab gel and avoid piercing the second-dimension gel with the strip. By convention, the acidic, or pointed, end of the IPG strip is on the left. The gel face of the strip should not touch the opposite glass plate.
- 10 Apply molecular weight marker proteins (optional): Apply the markers to a sample application piece in a volume of 15 to 20 l, then cover the piece with 50 l of agarose sealing solution. Pick up the application piece with forceps and place next to one end of the IPG strip. The markers should contain 0.2 to 1.0 mg of each component for Coomassie blue staining or about 10 to 50 mg of each component for silver staining.
- 11 Seal the IPG strip in place. For each IPG strip, melt an aliquot of agarose sealing solution in a heating block or boiling water bath. (Tip: An ideal time to carry out this step is during IPG strip equilibration.) Allow the agarose to cool slightly and slowly pipette the solution across the length of the IPG strip, taking care not to introduce bubbles. It will flow down between the glass plate and the support film and seal the IPG strip in place (see Fig 7-5). Agarose should also be used to seal any gap between the side of the gel and a side spacer. Allow a minimum of 1 min for the agarose to cool and solidify.

7.5 Loading the separation unit



WARNING! Switch the equipment off before opening the covers to avoid exposure to high voltage.

- 1 Once the electrophoresis tank has reached the desired temperature and the gels are ready, carefully slide the gels, one-by-one, into the tank. Until the buffer reaches the bottom of the rubber sealing tubes, the cassettes should be lubricated with buffer or water to prevent the rubber tubing from sticking to the cassettes. Once the buffer level reaches the sealing tubes, the gels should slide in easily.

Note: Forcing cassettes through the rubber tubes of the buffer seal without sufficient lubrication can damage the buffer seal.



Fig 7-5 Adding agarose sealing solution.

- 2 Fill any unused slots with the blank cassette inserts. When the last cassette is put into place, buffer will be pushed out of the lower tank into the upper tank via the two air vents at the corners of the sealing assembly. The final level of electrophoresis buffer in the upper tank should not be above the openings for the air vents.

Recommendation: For electrophoresis runs of six or fewer gels, it is helpful to alternate gel cassettes with blank cassette inserts. Alternating cassettes will make it considerably easier to remove the cassettes from

the unit following the run. The blank cassette inserts are easily removed first, leaving a larger gap that makes it easier to grasp and remove the gel cassettes.

7.6 Unloading and cleaning the separation unit

CAUTION! During all cleaning and draining operations the unit shall be disconnected from the mains power outlet. Any liquid on the equipment must be dried off before connecting the power.

- 1 After the run has been completed, remove one or more of the blank cassette inserts or gels and drain enough of the electrophoresis buffer from the tank to expose 2 to 3 cm of the gel cassettes. This will ease removing the remaining cassettes. When the first cassette, either blank or gel, is removed, a sucking sound will be heard as air is drawn into the lower chamber. For runs of six or fewer gels, alternating gel cassettes with blank cassettes also eases the removal of the gel cassettes at the end of a run. Leave enough of the electrophoresis buffer in the tank to act as a lubricant between the glass cassettes and the buffer seal.

There are two methods for removing the first cassettes from the unit: using (a) the cassette removal tool or (b) the hands.

A: Carefully insert the cassette removal tool between the cassette and the buffer seal, with the folded tip facing the cassette, until the tip is beneath the bottom edge of the cassette. Verify that the tool is caught on the bottom edge of the cassette, then lift it out slowly with the tool (Fig 7-6).



Fig 7-6 Using the cassette removal tools.

B: By hand, apply upward pressure alternately to each side of the cassette, gradually shifting it up until you can grasp it and remove it (Fig 7-7).



Fig 7-7 Removing cassettes by hand.

- 2 Open the cassettes using a Wonder Wedge (80-6127-88) and carefully transfer the gels to a staining tray (80-6468-17), for example. Take care to ensure that the gel does not adhere to the spacers.

Note: Vinyl gloves are less sticky than latex gloves and make it easier to handle large gels.

- 3 When all of the gels and blank cassette inserts have been removed, drain the buffer by turning the pump valve to drain with the pump on. Emptying will take about 1 min.
- 4 After the buffer has been removed, pour 3 to 4 l of distilled or deionized water into the unit and allow it to drain. Rinse the unit with 5 to 7 l of distilled or deionized water in circulate mode, empty again, and repeat if necessary.
- 5 Remove the lid from the unit by sliding it to the left and rinse it with distilled or deionized water. Slide the lid back on its hinges before using the unit again.
- 6 In most cases thorough rinsing is all the cleaning that is necessary. If a more thorough cleaning is required, see Section 12 for a detailed description of the removal of all the internal components.

8 Electrophoresis on pre-cast gels

Ettan DALT Gel, 12.5 is a pre-cast polyacrylamide gel for the second dimension of two-dimensional electrophoresis. The gel is cast onto a plastic support film. The gel size is 255 × 196 × 1 mm.

The gel is a homogeneous 12.5% polyacrylamide gel cross-linked with bisacrylamide. It is intended to be used in the Ettan DALT*twelve* System together with the Ettan DALT Buffer Kit. The gel is formulated for long shelflife and, when used with the buffer kit, generates a discontinuous buffer system yielding rapid runs with sharp, reproducible results. Performance and capacity of this gel and buffer system are similar to the widely used Laemmli (Tris-glycine) buffer system.

These instructions describe how to use Ettan DALT Gel, 12.5 together with the Ettan DALT Buffer Kit for the second-dimension of 2-D electrophoresis.

8.1 Ettan DALT Gel, 12.5 and Ettan DALT Buffer Kit

8.1.1 Package contents

Each gel package contains six gels and instructions. The buffer kit contains four bottles of buffer and 12 tubes of sealing solution. The solutions are sufficient for a single run of up to 12 gels.

Product	Quantity	Product number
Ettan DALT Gel 12.5%	6	17-6002-36
Instructions	1	71-5019-56
Ettan DALT Buffer Kit	enough to run 12 gels	17-6002-50
Anode buffer	1 bottle (75 ml)	
Cathode buffer	2 bottles (2 × 125 ml)	
Gel buffer	1 bottle (60 ml)	
Sealing solution	12 tubes (12 × 1 ml)	

Technical data

Gel composition	T = 12.5%, C = 3% (12.125% acrylamide, 0.375% bisacrylamide)
Separation range	Mr 12000 to 120000
Gel dimensions	255 × 196 × 1 mm
Buffer in gel	Piperidinopropionamide (PPA)*
Gel backing	Polyester film, 265 × 211 mm
Shelf life	6 months
Storage	4°C to 8°C
100 anode buffer	5 M diethanolamine (DEA), 5 M acetic acid
10× cathode buffer	0.25 M Tris, 1.92 M glycine, 1% (w/v) SDS
Gel buffer	Piperidinopropionamide (PPA)*
Sealing solution	Gel buffer with 0.5% agarose and 0.002% bromophenol blue

* The buffer system in this gel and buffer kit is covered by United States Patent 6,090,252 and others.

8.2 Description of the system

The Ettan DALT Gel, 12.5 gel is a pre-cast polyacrylamide gel for the second-dimension of large-format 2-D electrophoresis. It is bound to a plastic support film, which provides ease of handling and dimensional stability. The gel is intended for use in the Ettan DALT*twelve* System. The gel is inserted into a cassette that allows it to be run in a vertical mode with liquid buffers. The gel is used with the Ettan DALT Buffer Kit that includes concentrated buffers for running the gels, gel buffer for seating the gel in the pre-cast gel cassette, and sealing solution for attaching the IPG strip to the top of the slab gel.



Fig 8-1 Ettan DALT*twelve* Gel, 12.5 and buffer kit components.

The pre-cast gel cassette holds the Ettan DALT gel vertically in the Ettan DALT Electrophoresis Unit. It consists of a glass plate with spacers glued to the vertical edges and connected to a rigid plastic frame along one edge by a flexible hinge. The gel is placed against the glass plate between the spacers. When the cassette is closed and snapped together, the frame presses the gel evenly against the glass plate. The glass plate extends 5 mm higher than the plastic frame at the cathodic (–) edge providing a surface for sliding the IPG strip into position.



Fig 8-2 Pre-cast gel cassette.

The buffer in the gel is part of a unique buffer system that gives longer shelf life and shorter run times than the conventional Laemmli (Tris-glycine) system, while retaining the protein capacity and robustness of that system. Separations performed using Ettan DALT Gel, 12.5 are similar to those seen with a 12.5% Laemmli gel.

The associated buffer kit contains all the reagents necessary for a single run of up to 12 Ettan DALT Gel, 12.5 gels in the Ettan DALT*twelve* Electrophoresis Unit.

8.3 Preparing the Ettan DALT*twelve* Electrophoresis Unit



WARNING! Never overfill the vessel or spill liquid on the units due to the risk of electrical shock. Always make sure the equipment is disconnected from supply voltage when filling the tanks.

Ensure that the valve on the Ettan DALT Electrophoresis Unit is set to *circulate*. Fill the tank to the 7.5 l fill line with distilled or deionized water. Add the entire contents (75 ml) of the bottle of 100 anode solution from the buffer kit. Avoid pouring the 100× anode solution on the buffer seal tubing by spreading it slightly with one hand while pouring the solution (see Fig 8-3). Turn on the pump to mix. Set the unit to the desired temperature (25°C is recommended).



Fig 8-3 Addition of 100 anode to the separation unit solution.

8.3.1 Inserting the Ettan DALT Gel, 12.5 into the pre-cast gel cassette

- 1 Open the gel package. Cut around the gel on two sides at about 1 cm from the edge to avoid cutting the gel or the support film. Remove the gel from the package.

The gel is cast onto a plastic support film and does not cover the film entirely. The gel is covered with a protective plastic sheet. Markings on the protective sheet indicate the orientation of the gel and the direction of electrophoresis. The bottom (+ or anodic) edge of the gel is flush with the edge of the support film. The support film protrudes approximately 15 mm beyond the top (– or cathodic) edge of the gel and approximately 5 mm at either side.

- 2 Open an Ettan DALT Pre-cast Gel Cassette and place it on the bench top with the hinge down (see Fig 8-4).



Fig 8-4 Pre-cast gel cassette open.

- 3 Pipette 2 to 4 ml of gel buffer onto the centre of the glass plate (see Fig 8-5).



Fig 8-5 Gel buffer.

- 4 Remove the protective plastic sheet from the gel. Handling the gel only by the side support film margins, hold it, gel-side down, over the glass plate. Ensure that it is oriented with the cathodic (–) edge of the gel toward the cathodic (–) edge of the cassette. Flex the centre of the gel downward slightly and lower it toward the glass plate so that the middle of the gel contacts the puddle of gel buffer. The gel buffer will lubricate the gel and allow it to be moved and placed in the proper position (see Fig 8-6).



Fig 8-6 Initial placement of the gel.

- 5 Move the gel and allow it to fall against the glass so that the edges of the gel are against (not overlapping) the side spacers and so that the bottom (anodic) edge of the gel is flush (within 1 mm) of the bottom (anodic) edge of the glass plate. The protruding side support film margins (but not the gel) should rest on top of the side spacers (see Fig 8-7).



Fig 8-7 Final placement of the gel.

- 6 Use the roller to press out any bubbles and excess buffer from between the gel and the glass. Press firmly against the plastic support film with the roller and roll over the entire gel (see Fig 8-8). After rolling, the gel should adhere firmly to the glass and resist further movement.



Fig 8-8 Removing air bubbles.

- 7 Close the cassette and snap the plastic frame to the glass plate (see Figs 8-9 and 8-10).



Fig 8-9 Closing the cassette.



Fig 8-10 Snapping the cassette.

- 8 Repeat the procedure for each second-dimension gel to be run.

8.4 Applying the IPG strip

- 1 Leave the loaded gel cassette lying flat on the bench top with the glass plate down and the plastic frame up.
- 2 Rinse the IPG strip. Pour some of the diluted (1) cathodic buffer into a 100 ml graduated cylinder or similar vessel. Using forceps, remove the equilibrated IPG strip from the equilibration solution and dip it into the cathodic buffer in the cylinder. (This step lubricates the IPG strip and washes off any particulate material that may be precipitated on the surface of the IPG strip).
- 3 Place the IPG strip on the top (cathodic) surface of the gel. Holding one end of the IPG strip, carefully draw it across the shelf formed by the extension of the glass plate beyond the plastic frame, until the strip is completely on the glass plate and centered, gel face upward (see Fig 8-11).



Fig 8-11 Placing of the IPG strip. Push the IPG strip into place. Using a thin spatula or ruler, push against the plastic backing of the IPG strip to slide it a short distance into the gap between the glass plate and the support film and plastic frame. Be sure to push against the backing of the IPG strip, not the gel itself (see Fig 8-12). Place the cassette upright in the cassette rack with the glass plate forward. Continue to slide the IPG strip down until it contacts the surface of the second-dimension gel. The strip should just rest on the surface of the gel. Avoid trapping bubbles between the strip and the slab gel and avoid piercing the second-dimension gel with the strip. Note the orientation of the IPG strip relative to the gel (conventionally, the acidic [pointed] end of the IPG strip points to the left). The gel face of the strip should not touch the plastic support film.



Fig 8-12 Seating the IPG strip against the gel.

- 4 Seal the IPG strip in place. For each IPG strip, melt an aliquot of sealing solution from the buffer kit in a 100°C heating block or boiling water bath. (**Tip:** An ideal time to carry out this step is during IPG strip equilibration.) Allow the solution to cool slightly, then slowly pipette the solution across the length of the IPG strip, taking care not to introduce bubbles. It will flow down between the glass plate and the support film and seal the IPG strip in place (see Fig 8-13). There may be a gap of up to 2 mm between the edge of the gel and the side spacer. Any gap should be plugged by allowing some of the sealing solution to flow down the gap. Allow a minimum of 1 min for the agarose to cool and solidify).

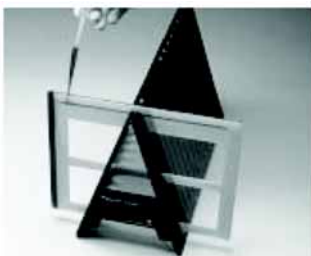


Fig 8-13 Sealing the IPG strip with agarose.

- 5 Repeat the procedure for each second-dimension gel to be run.

8.5 Inserting gels into the separation unit



WARNING! Disconnect the unit from the supply voltage when inserting gels.

- 1 When the lower tank buffer has reached the desired temperature, insert the loaded gel cassettes with the IPG strips in place (see Fig 8-14). Push blank cassette inserts into any unoccupied slots. Load the unit from back to front. Gel cassettes and blank cassette inserts slide much more easily into the unit if they are wet. Distilled or deionized water from a squirt bottle can be used to wet the cassettes and inserts as they are being loaded into the unit. When all 12 slots are filled, the buffer level should be slightly below the level of the buffer seal gaskets.



Fig 8-14 Loading gels into the separation unit.

- 2 Dilute the cathode buffer to working strength by adding both bottles of 10× cathode buffer (total volume 250 ml) to 2.25 l of distilled or deionized water.
- 3 Pour the diluted (1) cathode buffer into the top of the tank to the fill line. (Some of this buffer may drip through the gasket and mix with the lower anode buffer during the run, but this will not affect performance or results.)
- 4 Program the desired run parameters into the control unit, close the lid of the electrophoresis tank, and press start/stop to begin electrophoresis.

8.6 Detection

The Ettan DALT Gel, 12.5 gel can be stained or visualized with a variety of commonly used techniques, including Coomassie Blue and silver staining. When using the PlusOne Silver Staining Kit, Protein, a modified staining protocol should be used. Prepare the staining reagents (250 ml per gel) as indicated in the kit instructions with the following exceptions:

- Prepare twice the fixing solution called for (500 ml per gel rather than 250 ml).
- Prepare the developing solution with twice the formaldehyde called for (100 l per 250 ml of developing solution rather than 50 l). Stain the gels according to the following protocol:

Fixing	2 × 60 min*
Sensitization	60 min
Water wash	5 × 8 min
Silver	60 min
Wash	4 × 1 min
Developing	10 min†
Stop	60 min
Wash	2 × 30 min
Preserve	40 min

* The first fixing step may be prolonged up to 3 days if desired for the sake of convenience.

† Approximate time: this step may be visually monitored. The gels should be transferred to stop solution when the spots have reached the desired intensity and before the background staining becomes too dark.

9 Recommended running conditions

Gels	Step	Power (w/gel)	Approximate run Duration* (h:min)
Day runs (set temperature to 25°C)			
1 mm-thick gels	1	5	0:30
(lab-cast and precast)	2	17 (max 180)	4:00
1.5 mm-thick gels	1	5	0:30
	2	17 (max 180)	6:00
Overnight runs[†] (set temperature to 30°C and power supply to continuous run)			
1.0 mm-thick gels		1	17:00
1.5 mm-thick gels		1.5	17:00

* The time shown is approximate. Stop electrophoresis when the dye front is 1 mm from the bottom of the gel.

† For the best possible resolution, faster separation times should be used. Use the faster (< 6 h) protocols instead.

Note: The run times provided should only be used as guidelines or estimates. Decreasing the number of gels per run allows increased watts per gel, which reduces run times. The maximum power specified for each temperature is the limit of the cooling system capacity at that temperature.

10 Recipes



WARNING! Acrylamide is a neurotoxin. Always use mechanical pipettes and wear gloves when working with acrylamide solutions.

Note: Always adhere to local and national regulations when handling chemical substances.

Acrylamide stock (30.8%T)

	final conc.	amount
Acrylamide (MW 71.08)	30%	900 g
Bis (N,N' methylenebisacrylamide, MW 154.17)	0.8%	24 g
Distilled or deionized water		to 3 000 ml

May need filtration. Weigh acrylamide and bis in a hood; avoid contact with dust. Filter and store at 4°C.

1.5 M TrisCl, pH 8.8

	final conc.	amount
Tris (MW 121.14)	1.5 M	545 g
6 M HCl to pH 8.8		about 150 ml
Distilled or deionized water		to 3 000 ml

Adjust to pH 8.8 and store at 4°C.

10% (w/v) SDS

	final conc.	amount
Sodium dodecylsulphate (MW 288.38)	10%	10 g
Distilled or deionized water		to 100 ml

Store at room temperature.

10% (w/v) Ammonium persulphate

	final conc.	amount
Ammonium persulphate (MW 71.08)	10%	2 g
Distilled or deionized water		to 20 ml

Prepare fresh.

10% (v/v) TEMED

	final conc.	amount
TEMED (MW 116.2)	10%	0.5 ml
Distilled or deionized water		4.5 ml

Prepare fresh.

Displacing solution

(0.375 M TrisCl, pH 8.8, 50% (v/v) glycerol, bromophenol blue, 100 ml)

	amount
TrisCl (1.5 M, pH 8.8)	25 ml
Glycerol	50 ml
Bromophenol blue	2 mg
Distilled or deionized water	25 ml

Should be made fresh; stored solution may support microbial growth.

Water-saturated butanol

	amount
n or t-butanol	50 ml
Distilled or deionized water	5 ml

Combine in a bottle and shake. Use the top phase to overlay gels.
Store at room temperature indefinitely.

Gel storage solution

(0.375 M TrisCl, pH 8.8, 0.1% (w/v) SDS, 2 l)

	final conc.	amount
TrisCl (1.5 M, pH 8.8)	0.375 M	500 ml
10% (w/v) SDS	0.1% (w/v)	20 ml
Distilled or deionized water		to 2 000 ml

Store at 4°C.

10 SDS electrophoresis buffer

(250 mM Tris, 1.92 M glycine, 1% (w/v) SDS, approximate pH 8.3, 10 l)

	final conc.	amount
Tris (MW 121.14)	250 mM	303 g
Glycine (MW 75.07)	1.92 M	1440 g
SDS (MW 288.38)	1% (w/v)	100 g
Distilled or deionized water		to 10 l

Do not adjust the pH of this solution.

SDS equilibration buffer

(50 mM TrisCl, pH 8.8, 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, bromophenol blue, 200 ml)

	final conc.	amount
TrisCl (1.5 M, pH 8.8)	50 mM	6.7 ml
Urea (MW 60.06)	6 M	72.07 g
Glycerol (87% (v/v), MW 92.09)	30% (v/v)	69 ml
SDS (MW 288.38)	2% (w/v)	4.0 g
Bromophenol blue	trace	a few grains
Distilled or deionized water		to 200 ml

Store at -20°C. This is a stock solution. Add DTT or iodoacetamide before using.

Sealing solution

(0.25 mM Tris, 192 mM glycine, 0.1% (w/v) SDS, bromophenol blue, 0.5% (w/v) agarose, 25 ml)

	final conc.	amount
SDS electrophoresis buffer (see above)		25 ml
Agarose (IEF, NA, or M)		125 mg
Bromophenol blue	trace	a few grains

Combine all ingredients in a 250 ml Erlenmeyer flask. Swirl to disperse. On a low setting, heat in a microwave oven until the agarose is completely melted, about 1 min. Do not allow the solution to boil over.

Allow the agarose to cool slightly before using. Do not adjust pH.

10.1 Homogeneous gel solutions

900 ml

final %T	volume required for (ml)		
	10%	12.5%	15%
Acrylamide stock	300	375	450
1.5 M TrisCl, pH 8.8	225	225	225
Water	356	281	206
10% SDS	9	9	9
10% APS	9	9	9
10% TEMED	1.54	1.24	1.03

Note: The amounts of TEMED (0.025 to 0.09% (v/v)) and APS (0.1% (w/v)) suggested here are based on our experience. You may want to change volumes for your laboratory because of differences in temperature and reagent quality. Perform a small-scale test before using a new composition to check that your solution polymerizes in about 10 min.

The gel recipes are based on Laemmli, U.K. Nature 227, 680–685 (1970).

10.2 Gradient gel solutions

Light solution, 450 ml

final %T	volume required for (ml)				
	8%	10%	12%	14%	16%
Acrylamide stock	120	150	180	210	240
1.5 M TrisCl, pH 8.8	113	113	113	113	113
Water	207	178	148	118	88
10% SDS	4.5	4.5	4.5	4.5	4.5
10% APS	4.5	4.5	4.5	4.5	4.5
10% TEMED	0.96	0.77	0.64	0.55	0.48

Heavy solution, 450 ml

final %T	volume required for (ml)				
	12%	14%	16%	18%	20%
Acrylamide stock	180	210	240	270	300
1.5 M TrisCl, pH 8.8	113	113	113	113	113
Water	120	90	60	30	0
10% SDS	4.5	4.5	4.5	4.5	4.5
Glycerol	31	31	31	31	31
10% APS	2.3	2.3	2.3	2.3	2.3
10% TEMED	0.21	0.18	0.16	0.14	0.13

11 Troubleshooting

Symptom	Possible causes	Possible solutions
Electrical and mechanical		
No current at start of run	Insufficient volume of buffer in upper reservoir.	Ensure that the unit contains enough buffer to contact the upper electrode.
Buffer not circulating	Pump is not primed.	Turn circulation valve to drain to fill with buffer then back to circulate.
	Pump is off or set to auto. Pump is broken.	On control unit, set pump to on. Service call.
Display on the control unit blank	Unit is not turned on. Unit is not plugged in. Display is broken.	Turn unit on at power switch in back. Plug in unit. Service call.
Control unit display malfunctions		Turn the AC power switch off for a few seconds, then on again. If the problem persists, Service call.
Open-circuit warning	Safety interlocks not engaged.	Make sure that the lid is completely closed. Make sure that the pump valve is turned completely to circulate. Service call.
Gel casting		
Gel caster leaks		Apply a light film of GelSeal compound to the foam gasket before casting. Check the foam gasket for cracks or nicks and replace if necessary. If the stack is too thick, the front plate may not seat firmly against the gasket. Remove one or more of the filler sheets until the gasket seals.
Incomplete gel polymerization		Use only recent stocks of the highest-quality reagents. If the dry ammonium persulphate does not crackle when water is added to it, replace with fresh reagent. Use fresh ammonium persulphate. Solutions of extreme pH may not polymerize. Degas the monomer solution. Oxygen inhibits polymerization. Increase both ammonium persulphate and TEMED by 30% to 50%. Adjust the gel solution temperature to a minimum of 20°C.
Gel is too soft, too brittle, or white		Check and adjust crosslinker concentration. Standard SDS gels should have a crosslinker concentration of 2.6% ($\%C = (g \text{ bis} \times 100)/(g \text{ monomer} + g \text{ bis})$). Make up fresh acrylamide stock solution.
Gel exhibits swirls		If gel polymerized too fast (<10 min), reduce the concentration of catalyst (APS and TEMED) by 25%. If gel polymerized too slowly (>50 min), increase the concentration of catalyst (APS and TEMED) by 50%.
Dye front curves up "smiles"		Make up fresh acrylamide stock solution. Check circulation of the buffer. Pre-chill the buffer. Decrease power, voltage, or current.

Symptom	Possible causes	Possible solutions
Gel casting		
Gels cast simultaneously are different sizes		<p>Allow the solution to settle, or reach equilibrium, before adding the overlay.</p> <p>Add equal amounts of overlay solution to each gel.</p> <p>Add overlay as quickly as possible.</p>
Gradient gels—uneven layering		<p>Add sucrose (15% (w/v)) or glycerol (25% (v/v)) to the highpercentage monomer solution.</p> <p>Add a very small amount of bromophenol blue to the highpercentage monomer solution to track gradient formation.</p> <p>Note: Excessive bromophenol blue will inhibit polymerization.</p>
Unusually slow or fast run		<p>Check for leaks. All plates, spacers, and gaskets must be clean, dry, and free of grease.</p> <p>Make sure buffer is at the fill level and not covering the vent holes.</p> <p>Check the pH of the buffer. If the pH is exceeded, make fresh buffer; do not back-titrate.</p> <p>Check recipes, gel concentrations, and buffer dilutions. (For example, do not use TrisCl in place of Tris base for the electrophoresis buffer).</p> <p>Discard older acrylamide solutions and use only reagents of the highest quality.</p> <p>Only use freshly deionized urea of the highest quality.</p> <p>Adjust power, current, or voltage.</p>
Pre-cast gels		
Second-dimension separation proceeds slowly with high current	<p>All of the slots in the buffer seal are not occupied by either gel cassettes or blank cassettes.</p> <p>Anodic buffer has mixed with cathodic buffer from overfilling of either the cathodic or the anodic reservoir.</p>	<p>Ensure that all 12 slots in the buffer seal are occupied. Do not pour more than the suggested volume (7.5 l) into the lower reservoir.</p> <p>Ensure that the level of the anode (lower) buffer does not come above buffer seal when the separation unit is fully loaded. Remove any excess anode buffer from the upper reservoir. Ensure that the level of cathode buffer is not above the air vents in the upper reservoir.</p>
Dye front is irregular	<p>The top surface of the gel has been damaged during application of the IPG strip.</p> <p>Bubbles or liquid between the gel and the glass plate.</p> <p>Interfering substances in the first dimension.</p>	<p>Take care during application of the IPG strip that neither gel is damaged.</p> <p>Use the roller to remove any bubbles or excess liquid between the gel and the glass plate. Ensure that no visible bubbles remain and that the gel adheres firmly to the glass and resists movement.</p> <p>Contaminants in the sample can cause distortions or swollen regions in the IPG strip following IEF. Modify sample preparation to limit these contaminants. See 2-D Electrophoresis Using Immobilized pH Gradients—Principles and Methods (80-6429-60).</p>
Pronounced downward curving of the dye front on one side of the gel	There is an unfilled gap between the gel and one of the spacers.	When sealing the IPG strip into place, ensure that some of the agarose sealing solution flows down any gap that may exist between the gel and the spacer.

Symptom	Possible causes	Possible solutions
Stained gels		
Protein spots are diffuse or broader than usual		<p>Use only the highest-quality reagents.</p> <p>Make sure that polymerization is complete.</p> <p>Check equilibration time of IPG strips. Too long can lead to diffusion, and too short can lead to incomplete equilibration.</p> <p>Make sure the IPG strip rests on the slab gel surface without damaging.</p> <p>Problems with first dimension—see troubleshooting guides for IPG phor or Multiphor™ units, or 2-D Electrophoresis: Principles and Methods.</p>
Protein spots are poorly resolved		<p>Allow gel to polymerize completely.</p> <p>Begin electrophoresis as soon as the IPG strips are loaded, to prevent diffusion of low-molecular-weight proteins.</p> <p>Running too fast. Reduce the power, current, or voltage.</p> <p>Reduce the temperature setting.</p> <p>Problems with the first dimension.</p>
Protein spots are near the buffer front		<p>Pore size of the gel is too large. Increase the %T.</p> <p>Proteins degraded during sample preparation. Add protease inhibitors during sample preparation.</p> <p>Check the pH of the 4× gel buffer. It should be pH 8.8. Proteins will migrate faster below pH 8.8.</p>
Protein spots have not entered the gel when buffer front has reached the bottom of the gel		<p>The gel pore size is too small. Decrease the %T.</p> <p>Check the pH of the 4× gel buffer. It should be pH 8.8. Proteins will migrate more slowly above pH 8.8.</p>
Protein spots are at both extremes but not in center		<p>The molecular weight range of the sample requires an acrylamide concentration gradient to resolve the full range of proteins.</p>
Vertical protein streaks	IPG strip not properly placed on gel surface.	Make sure IPG strip uniformly contacts the gel surface along its entire length. Avoid gouging the surface of the separating gel.
Spots skewed or distorted	Gels run too fast – uneven migration.	Run at a lower power setting. Use a two-step program: Start at a low power setting until the proteins enter the gel, then increase the power for the remainder of the run.
	Uneven gel surface.	Overlay the running gel with water-saturated butanol before polymerization begins to avoid forming an uneven gel surface.
Heavy background after silver staining		Uneven gel polymerization or gradient formation.
		<p>Use reagents of the highest purity, preferably electrophoresis grade.</p> <p>Use deionized, double-distilled water.</p>

Symptom	Possible causes	Possible solutions
Distortion in the 2-D pattern	Bubbles between the gel and the glass plate.	Use the roller to remove any bubbles or excess liquid between the gel and the glass plate.
	Liquid between the gel and the glass plate.	Ensure that no visible bubbles remain and that the gel adheres firmly to the glass and resists movement.
	Interfering substances in the first dimension.	Contaminants in the sample can cause distortions or swollen regions in the IPG strip following IEF. These distortions can result in disturbances in the second-dimension.
Vertical gap in the 2-D pattern	Bubble between IPG strip and top surface of second dimension gel.	Ensure that no bubbles are trapped between the IPG strip and the top surface of the second-dimension gel.
Vertical streaking	Incorrectly prepared equilibration solution.	Prepare equilibration solution according to instructions.
	Poor transfer of protein from IPG strip to second dimension gel.	Use low power for sample entry phase. Extend entry phase if necessary.
	Insufficient equilibration	Prolong equilibration time.
Spots are vertically doubled, or "twinned"	IPG strip is not placed properly.	Ensure that the plastic backing of the IPG strip is against the glass plate of the second-dimension cassette.
Poor representation of higher-molecular-weight proteins	Incorrectly prepared equilibration solution.	Prepare equilibration solution according to instructions.
	Poor transfer of protein from IPG strip to second dimension gel.	Use low power for sample entry phase. Extend entry phase if necessary.



11.1 Recycling

This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of your equipment.

12 Care and maintenance

12.1 Cleaning



WARNING! When using hazardous chemicals, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the chemicals used. Follow local regulations and instructions for safe operation and maintenance of the system.



WARNING! During all maintenance the equipment shall be disconnected from the mains supply voltage.

For day-to-day operation of the unit, the cleaning procedure outlined in unit operation—thoroughly rinsing the separation tank with distilled or deionized water—is sufficient. If desired, the internal components of the separation unit can be removed for a more thorough cleaning (see below). The unit can also be periodically cleaned with a dilute solution of a mild detergent.

Clean the gel casting cassettes and pre-cast gel cassettes with a dilute solution of a laboratory cleanser such as RBS-35, from Pierce Chemical Company. Rinse the cassettes thoroughly with distilled or deionized water.

- DO NOT autoclave or heat any part above 50°C.
- DO NOT expose the unit or its parts to organic solvents, including >20% ethanol.
- If using radioactive reagents, decontaminate the unit with a cleaning agent such as CONTRAD 70 or Decon™ 90 from Decon Laboratories, Inc.

12.2 Replacing internal components

To remove the anode plate or any of the other internal components, follow the directions below.

- 1 Insert the buffer seal removal tools through the fifth or sixth slot, with the wider end underneath the tubes. Turn the tools 90° and place as close to the end blocks as possible. Carefully pull upward on the buffer seal until it is removed from the unit (see Fig 12-1).



Fig 12-1 Using the buffer seal removal tools

- 2 Slide the two baffle plates upward and out of the unit.
- 3 The two flow guides are removed by pulling out the retaining pins and lifting the blocks out.

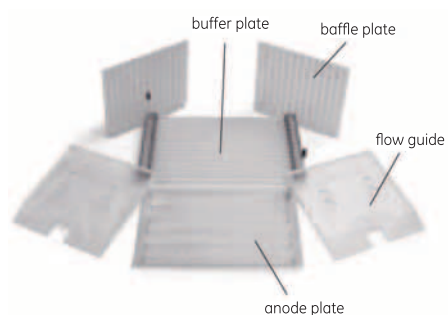


Fig 12-2 Internal components.

- 4 The anode plate then can be removed by unscrewing the flathead nylon screw and lifting the plate out.
- 5 To reassemble the unit, replace the anode plate and screw, making sure that the sealing sleeve is in place. Spread a small amount of GelSeal compound on the plug with a swab applicator to prevent corrosion.
- 6 Replace the flow guides and baffle plates.
- 7 Put a light film of GelSeal compound on the gasket of the buffer seal and reinsert it into the unit using even pressure. Make sure that it is fully seated before using the unit.

13 Customer service information

13.1 Technical service and repair

GE Healthcare offers complete technical support for all our products. If you have any questions about how to use this product, or would like to arrange to repair it, please call GE Healthcare Support.

IMPORTANT! Request a copy of the GE Healthcare “Health and Safety Declaration” form before returning the item. No items can be accepted for servicing or return unless this form is properly completed.

14 Ordering information

Product	Code number
Ettan DALT <i>twelve</i> Electrophoresis Unit and Power Supply/Control Unit	
110 to 120 V~	80-6466-46
220 to 240 V~	80-6466-27
Replacement Lid	80-6473-11
Replacement Buffer Seal	80-6473-30
Buffer Seal Removal Tool (2/pkg)	80-6474-63
Replacement Baffle Plate	80-6473-49
Replacement Flow Guide	80-6473-68
Replacement Anode Plate	80-6473-87
Ettan DALT Cassettes	
Pre-cast Gel Cassette	80-6466-65
Gel Casting Cassette, 1.0 mm	80-6466-84
Gel Casting Cassette, 1.5 mm	80-6488-69
Blank Cassette Insert	80-6466-03
Cassette Removal Tool (2/pkg)	80-6474-82
Ettan DALT Pre-cast Gels	
Pre-cast Gel, 12.5% (6/pkg)	17-6002-36
Buffer Kit (one run of 12 gels)	17-6002-50
Ettan DALT<i>twelve</i> Gel Caster	
Complete with Separator Sheets (16 pcs) and Filler Sheets (6 pcs)	80-6467-22
Separator Sheets (16/pkg)	80-6467-41
Filler Sheets (6/pkg)	80-6467-60
Black-Knobbed Screws (4/pkg)	80-6437-58
Triangular Sponge	80-6474-06
Acrylic Feed Tube	80-6437-20
Foam Sealing Gasket	80-6023-76
Silicon Tubing Set, two pieces/pkg: 9 mm o.d., 178 mm long; and 12.5 mm o.d., 16 mm long	80-6437-39
Replacement Tilt Leg with Nylon Screw	80-6474-25
Replacement Faceplate	80-6474-44

Product	Code number
DALT Gradient Maker with Peristaltic Pump	
115 V~	80-6067-65
230 V~	80-6067-84
Gradient Maker Gasket/Divider	80-6068-41
Gasket Adjuster Rod	80-6068-60
Knobs (4/pkg)	80-6437-58
Plastic Feed Tubing (1.8 m)	80-6068-03
Bow-Tie Mixer Kit	80-6068-22
Acrylic Feed Tube	80-6437-20
Accessories	
2-D Electrophoresis: Using Immobilized pH Gradients	80-6429-60
Cassette Rack (2/pkg)	80-6467-98
Equilibration Tubes (12/pkg)	80-6467-79
Stainless Steel Staining Tray Set	80-6468-17
GelSeal, 1/4 oz. tube	80-6421-43
Roller	80-1106-79
Fluorescent Rulers (2/pkg)	80-6223-83
Wonder Wedge Plate Separation Tool	80-6127-88
PlusOne Electrophoresis Chemicals and Reagents	
Urea, 500 g	17-1319-01
Dithiothreitol (DTT), 1 g	17-1318-01
Bromophenol Blue, 10 g	17-1329-01
Glycerol (87%), 1 l	17-1325-01
Acrylamide IEF (acrylic acid <0.002%), 1 kg	17-1300-02
Acrylamide IEF 40% solution, 1 l	17-1301-01
N,N',-Methylene bisacrylamide, 25 g	17-1304-01
N,N',-Methylene bisacrylamide 2% solution, 1 l	17-1306-01
Agarose IEF, 10 g	17-0468-01
N,N,N',N',-tetramethylethylenediamine (TEMED), 25 ml	17-1312-01
Ammonium Persulphate (APS), 25 g	17-1311-01
Tris, 500 g	17-1321-01
Glycine, 500 g	17-1323-01
Sodium Dodecylsulphate (SDS), 100 g	17-1313-01
Silver Staining Kit, Protein	17-1150-01
Molecular Weight Markers	
MW Range 2512 to 16949, 2 mg/vial, 1 vial	80-1129-83
MW Range 14400 to 94000, 575 mg/vial, 10 vials	17-0446-01
MW Range 53000 to 212000, 175 mg/vial, 10 vials	17-0615-01

For local office contact information, visit
www.gelifesciences.com/contact

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